

**PATENT COOPERATION TREATY**

From the Japan Patent Office  
 (INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY)

**PCT**

To: Agent of Applicant Hajime Takashima
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**WRITTEN OPINION OF THE INTERNATIONAL  
PRELIMINARY EXAMINING AUTHORITY**

(PCT Rule 66)

		Date of mailing (day/month/year) <b>05.4.2005</b>
Applicant's or agent's file reference <b>09624</b>		REPLY DUE      within    2    months from the above date of mailing
International application No. <b>PCT/JP2004/004612</b>	International filing date (day/month/year) <b>31.03.2004</b>	Priority date (day/month/year) <b>15.04.2003</b>
International Patent Classification (IPC) <b>Int. Cl<sup>7</sup> C12N5/06, C12N5/10, C12N15/09, A61K48/00, A01K67/027</b>		
Applicant <b>Kyoto University</b>		
<p>1. <input checked="" type="checkbox"/> The written opinion drawn up by the International Searching Authority is regarded a written opinion of the International Preliminary Examining Authority. <input checked="" type="checkbox"/> yes <input type="checkbox"/> no</p> <p>2. This <u>second</u> report contains indications relating to the following items:</p> <ul style="list-style-type: none"> <li><input checked="" type="checkbox"/> Box No. I Basis of the opinion</li> <li><input type="checkbox"/> Box No. II Priority</li> <li><input checked="" type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li> <li><input type="checkbox"/> Box No. IV Lack of unity of invention</li> <li><input checked="" type="checkbox"/> Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li> <li><input type="checkbox"/> Box No. VI Certain documents cited</li> <li><input type="checkbox"/> Box No. VII Certain defects in the international application</li> <li><input type="checkbox"/> Box No. VIII Certain observations on the international application</li> </ul> <p>3. The applicant is hereby invited to reply to this opinion.</p> <p>When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d). An extension is granted only when a rational reason exists and schedule is not full.</p> <p>How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.</p> <p>Also For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or argument, see Rule 66.4bis. For an informal communication with the examiner, see Rule 66.6.</p> <p>If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.</p> <p>4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: <u>15.08.2005</u></p>		

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PRELIMINARY EXAMINING AUTHORITY

Intern. application No.PCT/JP2004/004612

I. Basis of the opinion

1. Unless otherwise indicated under this item, this written opinion was drawn up based on the language in which the international application was filed.  
 This written opinion is in the following language \_\_\_\_\_ which is:  
 the language of a translation furnished for the purposes of the international search (under Rule 12.3 and 23.1(b)).  
 the language of publication of the international application (under Rule 12.4).  
 the language of a translation furnished for the purposes of the international preliminary examination (under Rule 55.2 and/or 55.3).
2. This opinion has been drawn up on the basis of (Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".):  
 the international application as originally filed.  
 the description: pages \_\_\_\_\_, as originally filed  
pages \_\_\_\_\_, received by the International Preliminary Examining Authority on \_\_\_\_\_  
pages \_\_\_\_\_, received by the International Preliminary Examining Authority on \_\_\_\_\_  
 the claims: Nos. \_\_\_\_\_, as originally filed  
Nos. \_\_\_\_\_, as amended under Article 19 PCT  
Nos. \_\_\_\_\_, received by the International Preliminary Examining Authority on \_\_\_\_\_  
Nos. \_\_\_\_\_, received by the International Preliminary Examining Authority on \_\_\_\_\_  
 the drawings: pages/Figs. \_\_\_\_\_, as originally filed  
pages/Figs. \_\_\_\_\_, received by the International Preliminary Examining Authority on \_\_\_\_\_  
pages/Figs. \_\_\_\_\_, received by the International Preliminary Examining Authority on \_\_\_\_\_  
 the sequence listing or related table  
see supplementary column relating to sequence listing.
3.  The amendments have resulted in the cancellation of:  
 the description, page \_\_\_\_\_  
 the claims, Nos. \_\_\_\_\_  
 the drawings, sheets/fig \_\_\_\_\_  
 the sequence listing (specify) \_\_\_\_\_  
 the table relating to sequence listing (specify) \_\_\_\_\_
4.  This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):  
 the description, page \_\_\_\_\_  
 the claims, Nos. \_\_\_\_\_  
 the drawings, sheets/fig \_\_\_\_\_  
 the sequence listing (specify) \_\_\_\_\_  
 the table relating to sequence listing (specify) \_\_\_\_\_

WRITTEN OPINION OF THE INTERNATIONAL  
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III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable have not been examined in respect of:

- the entire international application,  
 claims No. 18

because:

- the said international application, or the said claim No. 18 relate to the following subject matter which does not require an international preliminary examination (specify):

The claim is described the invention relating to a treatment method for human.

- the description, claims or drawings (indicate particular elements below) or said claims Nos. are so unclear that no meaningful opinion could be formed (specify):
- the claims or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

- no international search report has been established for the said claims No. 18

- the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C (guideline for preparing specification etc containing base sequence and/or amino acid sequence) of the Administrative Instructions In that:

the written form                    has not been furnished  
    does not comply with the standard

the computer readable form      has not been furnished  
    does not comply with the standard

- the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

- have not been furnished  
 do not comply with the technical requirements

- See separate sheet for further details.

WRITTEN OPINION OF THE INTERNATIONAL  
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V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step and industrial applicability;  
citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	<u>1-11, 14-16, 19-27</u>	YES
	Claims	<u>12, 13, 17</u>	NO
Inventive Step (IS)	Claims	<u>1-11, 14-16, 19-27</u>	YES
	Claims	<u>12, 13, 17</u>	NO
Industrial Applicability (IA)	Claims	<u>1-17, 19-27</u>	YES
	Claims		NO

2. Citations and Explanations

- Reference 1: Life Science Foundation (annual report), Heisei 14 edition (01 March, 2003) pp.17-19  
Reference 2: Cell.Mol.Life Sci., Vol.58, No.8, pp.1061-1066 (2001)  
Reference 3: Science, Vol.287, No.5457, pp.1489-1493 (2000)  
Reference 4: Gendai Kagaku Zokan 41, Saisei Igaku·Saisei Iryo (01 July, 2002) pp.24-28  
Reference 5: Proc.Natl.Acad.Sci.USA, Vol.98, No.23, pp.13090-13095 (2001)  
Reference 6: FEBS Lett., Vol.475, No.1, pp.7-10 (2000)

Claims 1-11, 14-16 and 19-27

The invention described in claims 1-11, 14-16 and 19-27 has novelty and an inventive step over the references cited in the International Search Report.

Reference 1 describes that even when using the cell differentiation suppressive factor LIF in the cultivation and maintenance of ES cells, it is troublesome to maintain an undifferentiated state while retaining the capacity of multiple differentiation, and that an investigation using the seminiferous tubule transplantation method has been ongoing to determine whether spermatogonia maintained and proliferated with the addition of GDNF (glial cell-derived neurotrophic factor) possesses the function for stem cells.

Reference 2 describes that GDNF and LIF, a member of the IL-6 family, control the self-proliferation and differentiation of stem cells in spermatogenesis.

Reference 3 describes that GDNF controls the self-proliferation and differentiation of undifferentiated spermatogonia, including spermatogonial stem cells.

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Supplementary column

Continued from column V

References 5 and 6 describe a method of producing a transgenic mouse by introducing an exogenous gene into spermatogonial stem cells using a retrovirus.

However, since *in vitro* proliferation of the cells, evaluated to be "spermatogonial stem cells" based on the measurements of stem cell functions such as colonization by seminiferous tubule transplantation, in a medium containing GDNF and LIF is not described and since, as described in the reference publication, (*Biology of Reproduction*, Vo.68, pp.2207-2214 (2003)), submitted by the Applicant, the technical common knowledge at the time of filing the present application denied growth of spermatogonial stem cells even with the use of GDNF or LIF, even those of ordinary skill in the art cannot easily envisage *in vitro* growth of "spermatogonial stem cells" in a medium containing GDNF and LIF".

Claims 12, 13 and 17

The invention described in claims 12, 13 and 17 lacks novelty over reference 4 cited in the International Search Report.

Reference 4 states that the spermatogenic capacity was acquired by transplanting spermatogonial stem cells to the testis.

(In the response dated February 14, 2005, it is argued that the "spermatogonial stem cells concerning claims 12, 13 and 17 are characterized in that they have been grown by culturing in a medium containing GDNF or an equivalent thereto and LIF". In Japan, however, since spermatogonial stem cells obtained not only by the culture method of the present invention but also by any method should be interpreted to be included in the scope of claims, the novelty cannot be acknowledged unless the cell can be expressly distinguished in the comparison with the "spermatogonial stem cells" described in reference 4 based on, for example, a specific cell surface marker and the like.)